REMARKS

Claims 1, 6, 9, and 18, as originally filed, are currently amended herein.

Claims 1 and 9 have now been amended to recite that the original extract obtained from biological material, by use of a fluid extractant comprising an aqueous isopropanol-KOH mixture or aqueous isopropanol, is one that contains a broad range of extracted chemical compounds. See Claim 1, steps (b) and (c), and Claim 9, step (c).

Support for these amendments is found throughout the Application (in the Background, the Summary, the Detailed Description, and the Examples and Drawings). See, e.g., Specification:

page 1, lines 27, 42, and 47;

page 2, line 5;

page 6, lines 7-8;

page 9, line 52 to page 10, line 36;

as well as the description of chemical compounds, partitioned into various fractions from the original extract, provided at, e.g.:

page 11, line 6, describing a first organic fraction thereof containing, e.g., pyridines, indoles, terpenes, phytols, alcohols, and hydrocarbons, and

page 11, lines 40-42, describing a second organic fraction thereof containing, e.g., fatty acids and phenols, and describing a second aqueous phase thereof containing, e.g., dicarboxylic acids, amino acids, sugars, and inorganic compounds.

Claim 1 has also now been amended to clarify, in steps (d) and (e), that "a chromatogram" includes one or more than one chromatogram, by replacing the word "a" with the phrase "at least one." Support for this amendment is found, e.g., in original Claim 9 and throughout the Specification.

Claim 9 has also now been amended to replace the terminology "alkylated" with the terminology "esterified," in step (K). Applicants note that this occurrence of the term "alkylated" resulted from an inadvertent oversight during preparation of this case. Both of these terminologies are found used interchangeably in the art in the context of describing alkylation of carboxylic acid compounds to produce esters therefrom, e.g., by reacting an alkyl alcohol with the carboxylic acid. Support for this amendment is found, e.g., in original Claim 9, step (G), to which antecedent the terminology of step (K) refers.

Claim 6 has now been amended to correct a typographical error reciting a dependency to "Claim 5." The recitation has now been corrected to read "Claim 2." Support for this amendment is found in original Claim 2, which recites the step (g) to which Claim 6 refers.

Claim 18 has now been amended to eliminate the vestigial recitation of a dependency to previously cancelled Claim 17. Claim 17 was cancelled during PCT Chapter II prosecution.

New Claims 19-21 have now been added.

New Claim 19 has been added to claim an embodiment of the subject matter of Claim 1 in which the extracts of the subject and control biological materials are first fractionated and then, in order to generate the chromatogram(s) of the extracts, at least one of the fractions is chromatographed. Support for new Claim 19 is found in original Claim 9 and throughout the Specification, e.g., see pages 11-14.

New Claims 20 and 21 have been added to claim embodiments of the subject matter of Claims 1 and 9, respectively, in which at least one of the subject and control chromatograms to be compared is an average or model chromatogram. Support for new Claims 20 and 21 is found, e.g., at page 6, lines 11-19 of the Specification.

The various items as numbered in the Action are addressed below.

Item 1. (A) Claims 1 and 9 stand objected for the presence of dark photocopy marks. Applicants supply herewith a List of Claim that lacks these photocopy marks.

(B) Claim 6 stands objected for dependency from Claim 5, rather than from Claim 2. Claim 6 has now been amended to replace the recitation of Claim 5 with "Claim 2."

(C) Claim 18 stands objected for dependency to previously cancelled Claim 17. Claim 18 has now been amended to delete the recitation of Claim 17.

Applicants believe that these amendments overcome the objections and respectfully request that they be removed.

Item 2. Claims 9-16 stand rejected under 35 U.S.C. 112, ¶2 for alleged indefiniteness in that Claim 9 recites, in step (K), "alkylated second fractions" whereas, in step (G), this Claim recites "esterified second fractions." Claim 9 has now been amended to correct this inadvertent wording problem that resulted in a lack of antecedent basis between step (K) and step (G). The term "esterified second fractions" is now recited in step (K).

Applicants also note that, throughout Claims 9-16, the term "biological material" occurs only in Claim 9, in the preamble and in steps (A), (B), (C), (K), and (L) thereof. In those occurrences in which the identities of the "subject" biological material and "control" biological material are not recited or otherwise indicated from context, it is intended that the term "biological material" refer to each of the two types of biological material, with the understanding that the operations specified in the claimed method for each of these two types of material can be performed either concurrently or separately in time.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be removed.

Items 3-4. No remarks are required.

Item 5. Applicants state on information and belief that the subject matter of the claims herein was commonly owned at the time the inventions were made.

Item 6. Claims 1-4, 6-8, and 18 stand rejected under 35 U.S.C. 103(a) for alleged obviousness over U.S. Patent No. 5,589,619 to Chappell et al. Applicants initially note that Claims 1, 6, and 18 have now been amended, with Currently Amended Claim 1 now reciting that the initial extract obtained by extraction of biological materials using aqueous isopropanol or aqueous isopropanol-KOH mixture, contains a broad range of chemical compounds.

The rejection notes that Chappell et al. teach:

- at col. 20, lines 20-27, that isopropanol and water-isopropanol mixtures are useful as solvents for extraction of plant pulp to dissolve or suspend squalene or sterol,
- in Example 3, that an "alcohol"/water-KOH solution was used to extract squalene, sterols, and sterol esters; that this solution was used to extract these compounds from transgenic leaf, root, and callus tissues; that these extracted compounds were analyzed by gas chromatography; and that the amounts of these compounds so analyzed were compared against corresponding control values.

However, Applicants note that Chappell et al. neither teach nor suggest that the selection of aqueous isopropanol (alone or in combination with KOH) offers particular advantages

compared to the other listed solvents: methanol, ethanol, acetone, acetonitrile, THF, hexane, chloroform, aqueous-organic mixtures, vegetable oils, and steam.

In contrast, Applicants have found that the selection of aqueous isopropanol (alone or in combination with KOH) offers particular unforeseen advantages vis-à-vis other solvents, in that the range of metabolites extracted is extremely broad, both in terms of chemical species and compound classes represented therein. Applicants have found that other solvents used in place of aqueous isopropanol (alone or in combination with KOH) do not permit the practical and efficient analysis of such a broad range of extracted metabolite compounds and compound classes.

For example, when Applicants compared the use of other solvents, e.g., methanol or ethanol, in place of isopropanol, the metabolite content of the resulting extracts was less optimal and performed in ways that made comparative analysis of broad classes of metabolites impractical. For example, these extracts did not offer the same range of diversity of metabolites as did the isopropanol-based extracts of the present invention. The chemical species present in the extracts also performed in subsequent fractionation so as to partition identical species and/or to partition members of the same compound class into more than one fraction. As a result, the chromatogram generated for each fraction was not quantitative for a given analyte or analyte class.

For example, a given fatty acid was, or various members of the fatty acid group were, found to variably partition into different fractions and thus appear on different chromatograms. In the case of methanol and ethanol use, different subpopulations of fatty acids were found to spontaneously esterify at different rates, and thereby result in variable partitioning during fractionation. (In contrast, the method of the present invention permits the fatty acids to partition together.)

The disadvantageous multiple-partitioning effect discovered with use of ethanol or methanol created the problem that direct subject-to-control chromatogram comparison did not provide reproducible, quantitative results for given compound species or classes. Further methods would, if possible, have had to be developed to *first* identify identical species in *different* chromatograms and then sum their peak values in order to subsequently permit straightforward quantitative comparisons against control values so as to identify significant differences between subject and control. As a result, were a non-isopropanol-based solvent used, at least some chemical species identification would likely have to be performed up-front

in the analysis in order to convert the chromatogram data into meaningful quantitative differences between subject and control. This would fail to achieve the efficient process of the present invention by which one may first obtain a quantitative comparison of species and classes by direct chromatogram comparison, thereafter employing a species identification method to further characterize the already-identified-as-different peaks.

Neither this problem nor its solution is identified in or overcome by the methods of Chappell et al. Thus, the method as taught by Chappell et al. would not have resulted in the simple, straightforward chromatogram comparison feature provided by the present invention, nor for such a broad range of metabolites.

Applicants note that, in contrast to the specific method of Chappell et al., the methods of the present invention, because of the breadth of metabolites extracted from biological materials, permit and make practical and efficient the practice of comparative metabolomics for identification of specific biochemical differences that result from differential treatment of subject versus control biological materials. As a result of the present invention, no specific test protocol need be formulated for metabolite analysis, such as the specific test protocol developed and used by Chappell et al. in order to identify differences in their particular targeted class of squalene and sterols.

Chappell et al. do not teach or suggest that -- aside from permitting extraction of this particular subclass of the hydrocarbons and mono-hydric polycyclic hydrocarbons -- any particular alcohol-, or aqueous alcohol-, or aqueous alcohol/KOH-based solvent will permit extraction of such a breadth of metabolites that a whole metabolome can be at least significantly represented in the resulting extract. Chappell et al. also do not teach how to obtain such a breadth of metabolites in such a way that fractionation thereof will result in species partitioning in such a manner that the resulting chromatograms may be directly compared to obtain quantitative results. Yet, these advantages are what Applicants have unexpectedly found results from selection of the use of aqueous isopropanol (alone or in combination with KOH). As a result, Applicants believe that one of ordinary skill in the art would not have reasonably expected to obtain the present invention, based on the cited reference.

Applicants do not disagree that GC/MS is a widely used technique for identification of chemical species present in a given sample or fraction, and that GC can be used to obtain chromatographs of mixtures of chemical species. Applicants do not disagree that various

orders of steps are considered useful and that computers are widely recognized as useful for data analysis, data comparison, and calculations. Yet, Applicants believe that the novel methods represented by combinations of these techniques with the particularly useful chromatograms obtained from the particularly useful extracts made according to the present invention, constitute methods that would not have been reasonably expected by one of ordinary skill in the art.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be removed.

Item 7. Claim 5, and Claim 18 depending therefrom, stand rejected under 35 U.S.C. 103(a) for alleged obviousness over U.S. Patent No. 5,589,619 to Chappell et al., as per the rejection set forth in Item 6 above, and further in view of U.S. Patent No. 6,544,566 to Waggle et al. Applicants initially note that Claim 1, from which Claim 5 depends, has now been amended to recite that the initial extract obtained by extraction of biological materials using aqueous isopropanol or aqueous isopropanol-KOH mixture, contains a broad range of chemical compounds.

The rejection states that Waggle et al. supply the teaching of an LC method (HPLC) to separate sterols, which is one of the teachings that Chappell et al. lacks in their sterol separation method. Applicants note that, in light of the remarks presented in Item 6 above, adding LC to the teachings of Chappell et al. fails to teach or suggest the methods of the present invention and fails to teach or suggest the advantages resulting from practice of the presently claimed methods.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be removed.

Item 8. Claims 9-16 have been held possibly allowable if rewritten or amended to overcome the rejection(s) asserted under 35 U.S.C. 112, ¶2, as set forth in Item 2 above. Claim 9 has now been amended to overcome this rejection. Applicants believe that these remarks and amendments overcome the rejection and respectfully request allowance of these Claims.

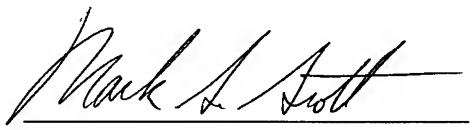
Item 9. No remarks are required.

CONCLUSION

Applicants believe that the above remarks and amendments overcome the objections and rejections presented in the June 10, 2003 Office Action. Applicants respectfully request that these objections and rejections be removed in light of these remarks and amendments, together with the teachings of the present Application.

Applicants request that if there are any issues remaining unresolved regarding any points raised by this Action or that if no Claims are found allowable after consideration of this response, that an interview by telephone or in person be granted, to be arranged for a mutually convenient time. Reconsideration and allowance of the Application are requested in view of the above.

Respectfully submitted,



Mark S. Scott
Registration No. 43,101
Phone: (517)636-1402

P.O. Box 1967
Midland, MI 48641-1967

Enclosures

- Additional Claims Fee Sheet, plus three (3) copies.